The Examiner rejected these Claims in an Office Action dated October 7, 2002, and in an Advisory Action dated January 17, 2003. These claims stand rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter that was not described in the specification is such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention and in such a way as to enable one skilled in the art to make and/or use the invention .

Claims 1-8, 10, 15, 18, 19, and 50-57 Are Fully Enabled And The Inventors Were In Possession of the Claimed Invention At the Time the Application Was Filed.

I. The Specification Teaches One Skilled in the Art How to Make the Claimed Compositions.

The Examiner previously acknowledged that the specification enables the preparation of crude cell lysates that can be tested for enzymatic cleavage activity (Office Action mailed October 7, 2002, page 3.) As noted by Applicants in their previous Response, the present application also provides detailed instructions for the making of *purified* preparations of the claimed compositions (see, *e.g.*, the paragraph in Example 3 on page 71 at lines 14-30.) The Examiner did not indicate in the Advisory Action mailed January 17, 2003 any remaining questions regarding this matter, which the Applicants understand to mean that the Examiner finds the teachings of the specification sufficient to teach one skilled in the art how to make the claimed compositions.

II. The Specification Teaches One Skilled in the Art How to Use the Claimed Compositions.

As described in their previously filed Response, Applicants have provided extensive teachings enabling one skilled in the relevant art to make enzymes comprising a heterologous functional domain, and enabling one skilled in the art to test any such enzyme for improved background specificity.

The Examiner previously acknowledged that Applicants have provided rapid screening assays for measuring enzyme activities in crude cell lysates (Office Action mailed October 7, 2002, page 3.) As indicated in their previously filed Response, Applicants also provided detailed description of how to use both crude lysates and purified enzymes in assays to measure cleavage activity on a variety of substrate cleavage structures. As previously noted, Example 1 includes detailed protocols for conducting cleavage assays, including examples of substrates, exemplary reactions conditions, and methods for detecting the products of the cleavage reactions. For example, as specified in Example 1 at page 57, lines 21-22, approximately 20 ng of each of

several purified mutant enzymes were used in the screens that generated the data presented in Tables 2-7 and in Figures 12, 14, 15, 19 and 25. In the paragraph immediately preceding, at page 57 lines 14-20, additional teachings of enzyme testing conditions are provided. The Examiner did not indicate in the Advisory Action mailed January 17, 2003 any remaining questions regarding this matter, which the Applicants understand to mean that the Examiner finds the teachings of the specification sufficient to teach one skilled in the art how to use the claimed compositions in cleavage assays.

III. The Specification Conveys to One Skilled in the Art That The Inventors Were In Possession of the Claimed Invention At The Time the Application Was Filed.

In the Advisory Action mailed January 17, 2003, The Examiner describes only one issue related to the rejections under 35 U.S.C. §112, first paragraph. The Examiner states that he did not conclude that the examples cited by the Applicants in the Response filed on December 9, 2002 showed that the heterologous functional domains provide the claimed improved background specificity. To clarify the examples previously provided, Applicants have included here the enzymatic activity measurements used to calculate improvements in background specificity. All data cited is from the Specification as filed. As described in more detail below, these data demonstrate that these examples do indeed show the improved background specificity indicated in the previous communication.

The Examiner admits that the specification provides support for the "improved background specificity" of the claimed compositions (Office Action mailed October 7, 2002, page 2.) As described in the Summary of the Invention (*e.g.*, on page 3, line 24 through page 4, line 3), improved background specificity relates to *an increased difference* between the detectable amount of cleavage of a specific structure (*e.g.*, IdT and IrT1 structures described on page 55 of the specification, which may be used to measure activity of any enzyme on DNA and RNA invasive cleavage structures, respectively), and the detectable amount of cleavage of any alternative structures such as might contribute to undesirable background in a particular assay (*e.g.*, the X and HP substrates depicted in Fig. 22 A and 22B). Improvements in the enzymes of the invention are generally determined by comparison of the function(s) of a test enzyme to the function(s) of a reference enzyme.

As further described on pages 3-4 of the present application, improved background specificity of the claimed enzymes may arise from a number of combinations of changes in the rate of cleavage of either or both specific and alternative structures. Improvement may arise, for example, from an increase in activity on a specific structure combined with a lesser increase, no increase or a decrease in activity on one or more alternative structures. Similarly, it may arise

from a decrease in activity on one or more alternative structures combined with a lesser decrease or no decrease in activity on a specific structure. Applicants have provided examples of enzymes showing improved background specificity by EACH of these combinations of activity changes.

Comparison of the turnover rates between a number of the test nucleases and reference nucleases are provided in Tables 2-7. The activity of these enzymes on specific structures is shown both as a cleavage rate (i.e., turnover rate) and as an indicated percentage of the activity of a reference nuclease. The comparison of the enzymes being screened or tested to reference or control enzymes is discussed in Example 1, at page 52 at lines 3-7. One skilled in the art, reading the Specification, would understand the columns indicated as "%Tth" and "%Taq 4M" as referring to the activity of the test enzyme indicated as a percentage of the activity of Tth enzyme (*i.e.*, Tth DN RX HT) and the Taq4M enzyme, respectively, tested under the same conditions. The activities for these reference enzymes on each of the test structures are provided in Table 2A and 2B, with Tth DN RX HT listed at the first entry in each table and Taq 4M as the sixth entry. "Taq 4M" is indicated parenthetically to be the abbreviated name for the enzyme listed as "Taq DN RX HT W417L/G418K/E507Q/H784A."

These examples of the claimed compositions were tested for cleavage of the desired structures (IrT1 and IdT) and for cleavage of the structures provided to test the generation of undesirable background cleavage (*e.g.*, HP and X structures). The turnover rates for a number of the test nucleases are provided in Tables 2-7. These turnover rates are compared to the turnover rates measured for the reference enzymes, Tth and Taq 4M. As noted by Applicants in their last communication, where the percentage of a reference activity for test enzyme on a particular substrate has not been provided explicitly (e.g., for the HP and X structures), it can be readily calculated from the turnover rates provided in these tables for the test and reference enzymes on these substrates. For the Examiner's convenience, the measurements and the calculated percentages of the reference activity have been included in table form (below) for each of the examples previously presented. Examples of the claimed compositions possessed by the inventors at the time of filing include (but are in no way limited to) the following:

1. **Taq 4M L109F/A110T (Table 3):** When L109F and A110T mutations were added to the Taq 4M variant, the activity on the IrT1 test substrate was measured as 2.45/min., or 92% of the activity the Taq 4M enzyme. The activity on the X structure was reduced to 68% of the X structure activity of Taq 4M. In addition, the activity on the HP structure was reduced to only 29% of that of the Taq 4M enzyme. For convenience, the turnover rates measured using this mutant enzyme and the reference enzyme on the indicated test

structures are shown in table form, below. The data for the Taq 4M reference enzyme are from Table 2 and the data for the mutant enzyme are from Table 3. The activity of the mutant enzyme expressed as a percentage of the activity of the reference enzyme on each indicated cleavage substrate is shown at the bottom of each column.

Structure:	IrT1	HP	X
Taq 4M	2.65	68.21	1100.18
Taq 4M L109F/A110T	2.45	19.71	749.69
% Reference activity:	92%	29%	68%

This improvement comprises reductions in activity on all of these structures, but the reduction of cleavage on the alternative HP and X structures is greater, thus background specificity is improved.

2. **Tth DN RX HT H786A/G506K/Q509K (AKK)(Table 2):** When H786A, G506K and Q509K mutations were added to the Tth DN RX HT variant, the activity on the IrT1 test substrate was increased to 179% of the activity the Tth enzyme on this substrate, while the activity on the HP structure was increased by a lesser amount, to 150% of the HP structure activity of the Tth enzyme. For convenience, the turnover rates measured using this mutant enzyme and the reference enzyme on the indicated test structures are shown in table form, below. These data are from Table 2. The activity of the mutant enzyme expressed as a percentage of the activity of the reference enzyme on each indicated cleavage substrate is shown at the bottom of each column.

Structure:	IrT1	HP
Tth DN RX HT	0.89	3.81
Tth DN RX HT H786A/G506K/Q509K (AKK)	1.59	5.7
% Reference activity:	179%	150%

This improvement comprises increases in activity on both of these structures, but the increase of cleavage on the alternative HP structure is smaller, thus background specificity is improved.

3. **Taq 4M R587A (Table 2)**: When an R587A mutation was added to the Taq 4M variant, the activity on the IrT1 test substrate was increased to 118% of the activity the Taq 4M enzyme, while the activity on the X structure was reduced to 23% of the X structure activity of Taq 4M. For convenience, the turnover rates measured using this mutant enzyme and the reference enzyme on the indicated test structures are shown in table form below. These data are from Table 2. The activity of the mutant enzyme expressed as a percentage of the activity of the reference enzyme on each indicated cleavage substrate is shown at the bottom of each column.

Structure:	IrT1	X
Taq 4M	2.65	1100.18
Taq 4M R587A	3.13	252.69
% Reference activity:	118%	23%

This improvement in background specificity comprises increased cleavage of a specific structure and decreased cleavage of the alternative X structure, thus background specificity is improved.

4. **Tth DN RX HT H786A/G203R (Table 5):** When the H786A and G203R mutations were added to the Tth DN RX HT variant, the activity on the IrT1 test substrate was increased to 180% of the activity the Tth enzyme on this substrate, while the activity on the X structure was reduced modestly to 97% of that of the Tth enzyme. This alone constitutes an improvement in background specificity. In addition, the activity on the HP structure was reduced to 55% of the HP structure activity of the Tth enzyme, which is an additional improvement in background specificity. For convenience, the turnover rates measured using this mutant enzyme and the reference enzyme on the indicated test structures are shown in table form below. The data for the reference enzyme is from Table 2 and the data for the mutant enzyme is from Table 5. The activity of the mutant enzyme expressed as a percentage of the activity of the reference enzyme on each indicated cleavage substrate is shown at the bottom of each column.

Structure:	IrT1	HP	X
Tth DN RX HT	0.89	3.81	101.9
Tth DN RX HT H786A/G203R	1.61	2.1	98.8
% Reference activity:	180%	55%	97%

As detailed above, the data presented in the present Application and summarized in the Response filed on December 9, 2002 clearly show that each of these examples describes an enzyme having heterologous functional domains and having *an increased difference* between the detectable amount of cleavage of a specific structure (*e.g.*, IdT and IrT1 structures described on page 55 of the specification, which may be used to measure activity of any enzyme on DNA and RNA invasive cleavage structures, respectively), and the detectable amount of cleavage of at least one alternative structure such as might contribute to undesirable background in a particular assay (*e.g.*, the X and HP substrates depicted in Fig. 22 A and 22B). These cited examples of the claimed compositions therefore each demonstrate the "improved background specificity" of the claimed compositions.

The subject matter of Claims 1-8, 10, 12-15, 18, 19 and 50-57 is described in the specification is such a way as to enable one skilled in the art to make and use the invention. Specifically, the Application provides extensive teachings on how to make and use enzymes having a heterologous functional domain providing improved background specificity. Further demonstrating that these teachings are enabling, Applicants provide data showing possession at the time of filing of numerous examples of enzymes created by the disclosed methods, such enzymes providing the claimed improved background specificity. As such, Applicants assert that the present application clearly satisfies the requirements of 35 U.S.C. §112, first paragraph cited by the Examiner as his basis for this rejection. Applicants respectfully request that this rejection be withdrawn.

CONCLUSION

For the reasons set forth above, it is respectfully submitted that Applicants' claims should be passed to allowance. Should the Examiner believe that a telephone interview would aid in the prosecution of this application, Applicants encourages the Examiner to call the undersigned collect at (608) 218-6900.

Dated: April 7, 2003

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TABLE 3: Rational arch mutations

DNA activity table

	IdT	%Tth	%Taq4M	HP	X
Taq 4M P88E/P90E	10.20	32%	27%	2.00	97.00
Taq 4M G80E	26.30	82%	69%	103.6	2900
Taq 4M L109F/A110T	36.45	114%	95%	19.71	749.69

RNA activity table

	IrT1	%Tth	%Taq4M
Taq 4M P88E/P90E	0.10	11%	4%
Taq 4M G80E	3.11	349%	117%
Taq 4M L109F/A110T	2.45	275%	92%